Alteration of Masquelet’s induced membrane characteristics by different kinds of antibiotic enriched bone cement in a critical size defect model in the rat’s femur

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ABSTRACT

The Masquelet technique for the treatment of large bone defects consists of a 2-stage procedure. In the first stage, a polymethylmethacrylate (PMMA) cement spacer is inserted into the bony defect of a rat’s femur and over a period of 2–4 weeks a membrane forms that encapsulates the defect/spacer. In a second operation the membrane is opened, the PMMA spacer is removed and the resulting cavity is filled with autologous bone. Different kinds of bone cements are available, with or without supplemental antibiotics. Both might influence the development and the characteristics of the induced membrane which might affect the bone healing response. Hence, this comparative study was performed to elucidate the effect of different bone cements with or without supplemental antibiotics on the development of an induced membrane in a critical size femur defect model in rats.

A total of 72 male SD rats received a 10 mm critical size defect of the femur which was stabilised by a plate osteosynthesis and filled with either Palacos + Gentamycin, Copal Gentamycin + Vancomycin, Copal + Gentamycin + Clindamycin or Copal Spacem. The induced membranes were analysed after two, four and six weeks (wks) after insertion of the cement spacers (n = 6/group). Paraffin embedded histological sections of the membrane were microscopically analysed for membrane thickness, elastic fibres, vascularisation and proliferation by an independent observer blinded to the group setup.

The thickness of the induced membrane increased significantly from 2 wks (553 μm) to 6 wks (774 μm) in group Palacos + Gentamycin whereas membrane thickness decreased significantly in groups Copal + Gentamycin + Clindamycin (682–329 μm) and Copal Spacem (916 μm to 371 μm). The comparison between the groups revealed significantly increased membrane thickness in group Palacos + Gentamycin and Copal Gentamycin + Vancomycin in comparison to group Copal + Gentamy- cin + Clindamycin six weeks after induction. However, the fraction of elastic fibres was significantly increased in groups Copal + Gentamycin + Clindamycin (71%, 80%) and Copal Spacem (82%, 81%) after 2 and 4 weeks in comparison to the groups Palacos + Gentamycin (56%, 57%) and Copal Gentamycin + Clindamycin + Vancomycin (63%, 69%). Those differences however were partly diminished after 6 wks. The ratio of immature (vWF+) to more mature (CD31+) blood vessels increased significantly in groups Palacos + Gentamycin and Copal Gentamycin + Vancomycin whereas no significant alterations were noted in groups Copal + Gentamycin + Clindamycin and Copal Spacem.

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Introduction

Large bone defects frequently occur in the course of trauma, osteomyelitis or due to tumour resection. Gold standard for the treatment of large bone defects is transplantation of autologous bone grafts, preferably taken from the iliac crest. However, bone volume at the donor site is limited and the surgical procedure for bone extraction is often accompanied by donor site morbidities such as long-lasting pain [1,2].

A variation on these treatments, first described by Alain Masquelet [3,4] consists of a 2-stage procedure in which a membrane is induced and forms around the bone defect. In the first step, a polymethylmethacrylate (PMMA) cement spacer without any additives such as antibiotics is inserted into the bony defect and over a period of 6–12 weeks a foreign-body reaction causes a membrane that encapsulates the defect/spacer. In a second operation the membrane is opened, the PMMA spacer is removed and the resulting cavity is filled with autologous bone. Using this technique, Masquelet, and others have described complete bone healing and restoration of limb function in several clinical cases [3,5].

Masquelet et al. and separately Viateau et al. described these induced membranes as being 1–2 mm thick with histological properties similar to those of synovial tissue “providing an environment favourable for bone healing”. They continue to describe the membrane as “contributing a rich vascularisation and inhibiting soft tissue invasion into the bony defect that may potentially protect autologous bone grafts from resorption” [3,6]. We compared cellular constitution, histological changes, and growth factor expression in induced membranes to periosteum 2, 4 and 6 weeks after induction. We found that membranes formed around bone defects were significantly different from periosteum with regard to structural characteristics, location of blood vessels and overall thickness. Membranes induced at the femur’s defect (2 wks only) contained MSC (STRO-1) and the mediators BMP-2, TGF-β and VEGF. We further observed that osteogenic and neovascular activity had mostly subsided by 6 weeks in induced membranes [7].

Bone cements are often prepared with antibiotics in order to control local infections at the operating field in orthopedic and trauma surgery. This procedure leads to locally higher antibiotics’ concentrations compared to those following oral or intravenous administration [8]. “Gentamycin is the most frequently used antibiotic for loading bone cement because it has a broad antimicrobial spectrum and it can withstand the high temperatures reached during polymerisation of the bone cement” [9]. Common antibiotics which are qualified for incorporation into bone cements are clindamycin, gentamycin and vancomycin. It has been shown that those antibiotics exert cytotoxic effects and impair cellular differentiation in high dosages, with clindamycin as the most toxic, followed by vancomycin and gentamycin [8,10–12].

The purpose of this study was therefore to characterise the assembly of the induced membranes in dependency on the antibiotics added to the bone cement, specifically as it relates to osteogenic and angiogenic/vasculogenic properties that contribute to bone healing. This was achieved by measuring vascularity, the presence and proliferation of putative bone forming cells (MSC), and histological analysis in a rat’s femur critical size defect model.

Materials and methods

Animal care

All animal experiments were performed in accordance with regulations set forth by our institution’s animal care and oversight committee (Project No. F3/24; Regierungspräsidium, Darmstadt, Germany) in accordance to German law. Seventy-two 12-week old male Sprague-Dawley rats (Janvier, France), weighing approximately 350–400 g were housed in individual cages, in temperature (21 °C), air flow and light (12 h day and 12 h night) controlled rooms and received rat food and water ad libitum. Animals were monitored daily in the postoperative period for signs of pain, discomfort and complications.

Cements

The following commercially available and clinically approved cements were used: Palacos R + G, supplemented with gentamycin (20 g cement powder contain 0.25 g gentamycin), Copal G + V, supplemented with vancomycin (43 g cement powder contain 0.5 g gentamycin + 2 g vancomycin), Copal G + C, supplemented with gentamycin and clindamycin (42.7 g cement powder contain 1 g gentamycin + 1 g clindamycin) and Copal Spacem without antibiotics but with a calcium carbonate additive.

All cements are based on the same polymethylmethacrylate (PMMA) formula and were purchased from Heraeus Medical (Wehrheim, Germany).

Surgical procedure

Treatment groups (see Table 1).

Under general intraperitoneal anaesthesia (Ketavet 70 mg/kg and Rompun 10 mg/kg) the right leg was shaved, cleaned and disinfected and animals were placed in a lateral position. A longitudinal incision was made in the skin and the fascia over the right femur. The biceps femoris and vastus lateralis muscles were separated bluntly exposing the antero-lateral aspect of the femoral bone. A 6-hole, 1.5 mm stainless-steel mini-plate (Synthes, Dubendorf, Switzerland, CompactHand) was applied to the anterior aspect of the femur shaft and secured in place with two proximal and two distal 1.5 mm cortical screws (Synthes, CompactHand). After stabilisation, a critical size defect (CSD), measuring 10 mm, was created in the femur bone shaft, underneath the plate using a Gigli saw (RI-Systems, Davos, Switzerland). The cement was hand mixed according to the manufacturer’s protocol. The bone defect was then filled with the respective PMMA cement and moulded into a cylindrical shape (Fig. 1). The wound was irrigated with sterile saline, the fascia was re-approximated with interrupted 5-0 Vicryl sutures, and the superficial fascia and skin closed with Prolene 5-0 suture (Ethicon, Germany). Animals were returned to their cages, monitored daily for the occurrence of abnormal behaviour or complications and analgesia was given for 5 days postoperatively.